the yucca moths. Importantly, the evolution of obligate pollination behaviour could evolve in the moths during a period of coexistence with less effective ancestral pollinators and without any counteradaptation in the host. ☐

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Word recognition in the human inferior temporal lobe

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STUDIES of primates[1] and of patients with brain lesions[2] have shown that the visual system represents the external world in regions and pathways specialized to compute visual features and attributes. For example, object recognition is performed by a ventral pathway located in the inferior portion of the temporal lobe[3]. We studied visual processing of words and word-like stimuli (letter-strings) by recording field potentials directly from the human inferior temporal lobe. Our results showed that two discrete portions of the fusiform gyrus responded preferentially to letter-strings. A region of the posterior fusiform gyrus responded equally to words and non-words, and was unaffected by the semantic context in which words were presented. In contrast, a region of the anterior fusiform gyrus was sensitive to these stimulus dimensions. These regions were distinct from areas that responded to other types of complex visual stimuli, including faces and coloured patterns, and thus form a functionally specialized stream within the ventral visual pathway.

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Within the ventral visual pathway, regions specialized for processing faces[4][7] and colours[8] have been identified in monkeys. In humans, imaging studies using positron emission tomography (PET)[9][12] and intracranial recordings of event-related potentials (ERPs)[13,14] have localized these functional areas in regions of extrastriate cortex. Studies of brain lesions[15][17] and cortical stimulation[18] in patients suggest that there is a specialized region for the processing of written words in the inferior temporal lobe. One PET study[19], but not another[20], reported increased blood flow in the inferior temporal lobe during reading. We investigated the role of the inferior temporal lobe in word recognition by recording cortical surface ERPs directly from the inferior temporal and occipital lobes in 27 patients (381 electrode locations overall) while they performed tasks involving visually presented letter-strings, faces and control stimuli (Fig. 1).

Two types of ERPs elicited by letter-strings were recorded in the fusiform gyrus. Large negative field potentials with peak latencies near 200 ms (N200) were elicited in discrete regions of
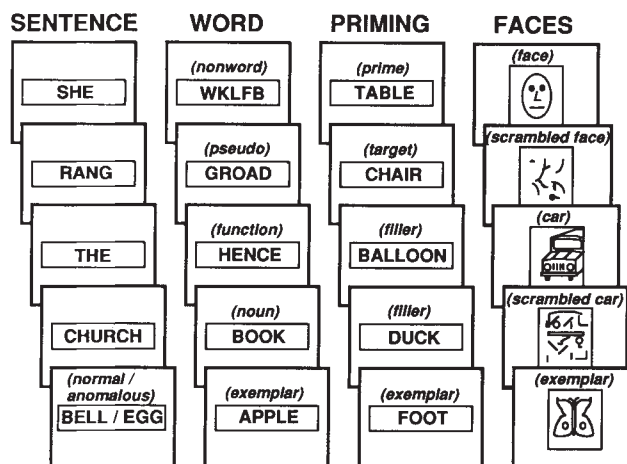

FIG. 1 Behavioural tasks. Twenty-seven patients (13 women, 23 right-handed, 18–55 years of age) with medically intractable epilepsy, who were being evaluated for possible neurosurgery[27], viewed a computer monitor placed 60 cm from the eyes. Language lateralization was tested using the sodium amytal procedure in 22 patients. All were left-hemisphere dominant. In the sentence task[28], words in each of 160 sentences were presented briefly (100 ms duration) and rapidly (500–800 ms stimulus-onset asynchrony, SOA). Half the sentences terminated with a semantically correct best completion, thus the normal terminal word was primed by sentence context. Words or letter-strings subtended visual angles of 3–10° horizontally and 0.5–1.0° vertically. Patients read the sentences silently for comprehension and were told that some endings might appear odd. Debriefing ensured that patients read the sentences. In the word and priming tasks[22], 220 stimuli were presented (500 ms duration, 2.0–2.1 s SOA). In the word task, patients detected names of fruits and vegetables (10% of the stimuli). The remaining letter-strings were equally divided among non-pronounceable non-words, pronounceable non-words (pseudowords), words serving grammatical function, and concrete nouns. In the priming task, patients detected names of body parts (10% of the stimuli). The remaining words were all concrete nouns. Half were pairs of semantically related nouns, with the first word of each pair designated the prime and the second word the target. The remaining half were unrelated word pairs (fillers). The face task required catagorization of stimuli (250 ms duration, 1.8–2.0 s SOA) subtending 7.2 × 7.2° of visual angle[14]. Patients detected pictures of butterflies (10% of the stimuli). The remaining stimuli were equally divided among faces, digitally scrambled faces (equiluminant with the original stimulus but unrecognizable as faces), cars and scrambled cars. Other control stimuli included black–white or coloured checkerboards. The requirement to make key presses to exemplars ensured the patient's attention to the stimuli and provided another measure of ERP selectivity to category-specific stimuli.

the posterior fusiform gyrus by all types of letter-strings but not by other stimuli (Fig. 2). Letter-string type (locations 1 and 2) and semantic priming (locations 3 and 4) did not affect N200. The exact stimulus attributes necessary to generate letter-string N200s are unknown, but at most locations they are not generated by faces (location 1), complex biological (location 2) or non-biological (location 4) objects and by checkerboards (location 3).

The second type of ERP elicited by letter-strings was recorded in the anterior portion of the fusiform gyrus (Fig. 3). This ERP was a broad positive potential with a peak near 400 ms (P400).

In marked contrast to the posterior N200, the anterior P400 was sensitive to properties of letter-strings. Words with semantic content elicited larger P400s than words with grammatical function (location 2). Semantic priming by pre-exposure to a related word (locations 3 and 4) or by sentence context (locations 1 and 3) diminished or abolished P400. P400 was not elicited by non-words (location 4) or by a variety of control stimuli including checkerboards (location 1) and faces (location 2). Electrodes placed inside the anterior temporal lobe near the cortical surface electrodes recorded a broad negative ERP with peak latency and waveshape equivalent to P400 (refs 21, 22). Such polarity inversions[23] indicate that P400 is locally generated in the anterior fusiform gyrus.

The anatomical and functional specificity of N200 and P400 is illustrated for a single patient in Fig. 4a. A letter-string N200 (location 1, solid arrow) was recorded medially and posteriorly to a face N200 (ref. 14; location 3, curved solid arrow); this spatial differentiation was seen in six of eight patients in whom
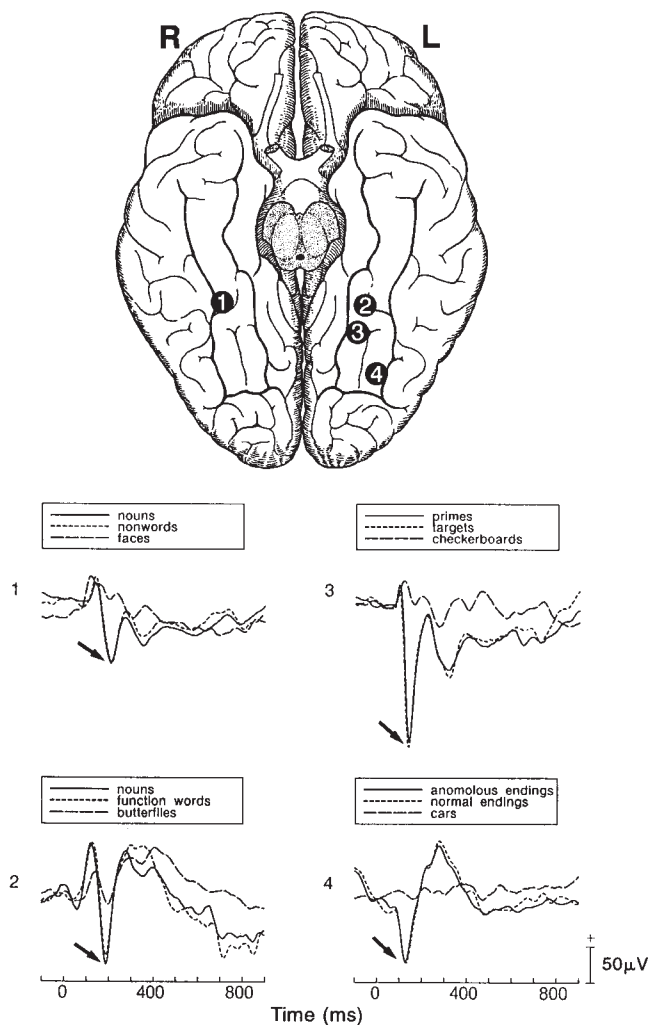

FIG. 2 Examples of letter-string N200s (arrows) in posterior fusiform gyrus. Strips of 8–12 electrodes (2.2 mm diameter, 1.0 cm interelectrode distance) were placed on the cortical surface of the brain for 3–21 days. The electroencephalogram was recorded simultaneously from 32–64 electrodes referential to a mastoid (250 Hz digitization rate; 10,000 grain; 0.1–100 Hz band pass). EEG segments were segregated by stimulus type and averaged. Electrodes were localized by post implant magnetic resonance images. Electrode locations are depicted using gyral anatomy in the lateral axis. Coordinates of Talairach and Tournoux[29] were used for the anterior–posterior axis. ERPs from four patients are depicted (locations 1–4). In each example, large N200s were elicited by letter-strings (nouns, non-words, function words, targets, normal and anomalous sentence endings) but not by control stimuli (faces, butterflies, high-contrast checkerboard patterns, and cars). Within patients, the amplitudes and latencies of the letter-string N200s did not vary with the properties of the letter-strings. Waveforms on location 3 are from a left-handed patient. All other data in this and subsequent figures are from right-handed patients.
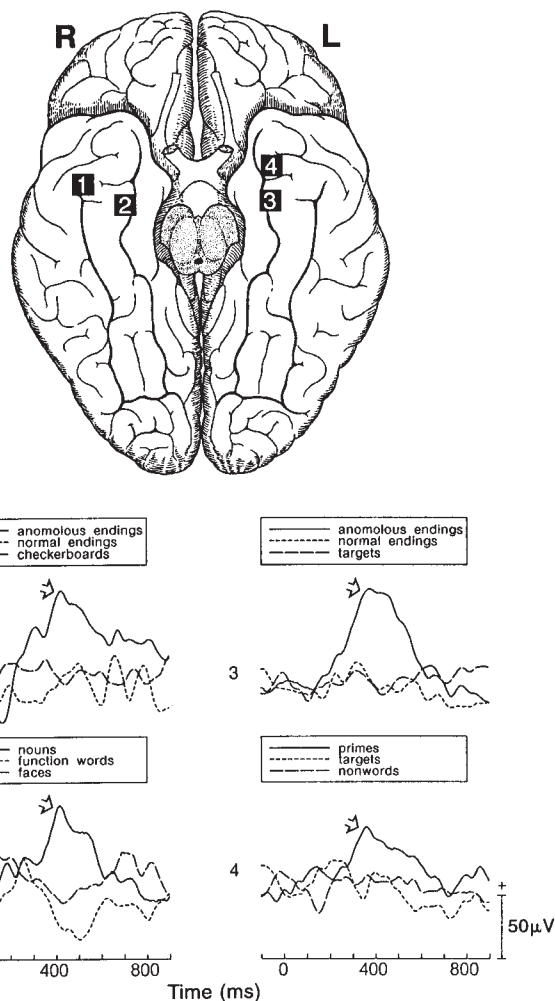

FIG. 3 Examples of letter-string P400s (arrows) in anterior fusiform gyrus. ERPs from four patients are depicted (locations 1–4). In each example, large P400s were elicited by nouns (anomalous sentence endings, locations 1 and 3; and unprimed nouns, locations 2 and 4) but not by control stimuli (checkerboards, location 1; faces, location 2). P400 was sensitive to the properties of letter-strings and to the context in which nouns were presented. Semantic priming (targets, locations 3 and 4) or priming by sentence context (normal sentence endings, locations 1 and 3) diminished or abolished P400. Function words (location 2) and non-pronounceable non-words (location 4) did not elicit P400.
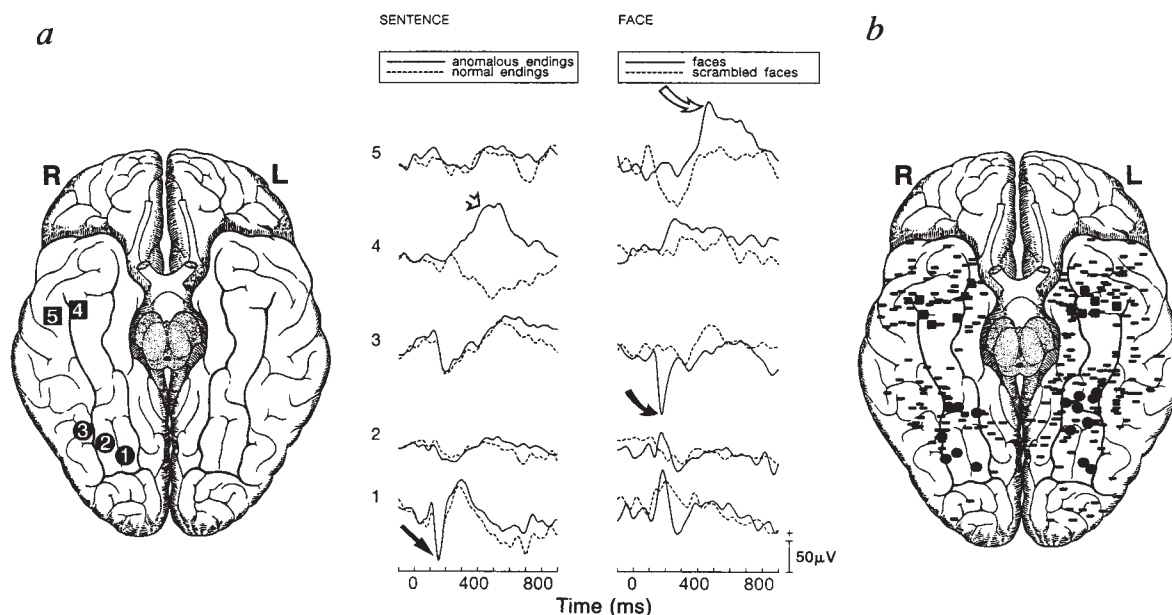
FIG. 4 Modularity and modulation of letter-string ERPs. *a*, Recordings from the sentence task are compared with recordings from the face task in a patient. A letter-string N200 (arrow) was observed at electrode location 1 in the posterior fusiform gyrus. In contrast, location 3 of the same electrod strip recorded a face N200 (curved arrow) in the inferior temporal gyrus. A letter-string P400 (open arrow) was elicited at location 4 in the anterior fusiform gyrus, while a face P400 (curved open arrow) was elicited at location 5 in the anterior inferior temporal gyrus. The letter-string N200 was not sensitive to priming in sentences, whereas the letter-string P400 was markedly reduced. As in previous reports[14,24], the face N200 and P400 were specific to faces. The sensitivity of the face P400 to context is at present unknown. *b*, Electrode locations of letter-string N200 (●), letter-string P400s (■), and locations where letter-strings did not elicit selective ERPS (−) are shown for all patients. Mean Talairach locations[29] were: N200 left, $X = $G.18, $Y = $lc.05; N200 right, $X = $H.03, $Y = $rc.00; P400 left, $X = $E.21, $Y = $lb.46; P400 right, $X = $E.43, $Y = $rc.17.

N200s were recorded both to letter-strings and faces. Similarly, P400s elicited by words (open arrow) and faces[24] (curved open arrow) were recorded from separate locations (4 and 5) in the anterior temporal lobe. The locations from which all letter-string N200s and P400s were recorded are summarized in Fig. 4*b*. Both letter-string and colour recordings[13] were obtained in five patients; in all cases N200s were generated by letter-strings at locations lateral to those that generated the largest colour ERPs.

These results suggest that there is a separate stream specialized for word recognition within the ventral visual pathway, and that a sequence of processing occurs over a period of ∼200 ms. Two putative nodes of the word stream appear to compute increasingly higher-order characteristics of words. In discrete regions of the posterior fusiform gyrus, N200s are elicited by all types of letter-strings, and may reflect the formation of a word percept. Important stimulus features for processing in the word–percept node may be the characteristic configuration of small elements arranged in a linear array, the requirement for integrating multiple elements to yield an object[17], or the grouping of letters into recognizable word forms[25]. Given their insensitivity to word type, letter-string N200s probably reflect pre-lexical processing of words or possible words. This pre-lexical node is postero-lateral to a possible lexical region of the left inferior medial temporal lobe activated in PET studies by real words and pronounceable non-words[19].

In discrete regions of the anterior fusiform gyrus, P400s are sensitive to semantic content and context, and thus may correlate with the activation of a word concept. The word–concept node may form visual or multimodal image-based representations of words or may serve a mnemonic function in associating a stimulus with its context, emotional valence or verbal label[22]. A mnemonic role is consistent with the proximity of the anterior fusiform region to entorhinal cortex, hippocampus and amygdala. The fusiform gyrus is one of several structures often damaged in alexic and agnosic patients[15-17]. Damage to the word-recognition stream in the fusiform gyrus may help to explain some forms of reading and object-naming deficits in patients with brain lesions[2,15-17], such as the posterior or occipital form of alexia[26].

Across patients, the amplitudes and latencies of N200 and P400 were similar in both hemispheres, suggesting that the word processing described here is bilateral. However, electrodes were rarely placed symmetrically in the two hemispheres, making it difficult to assess laterality. Additional nodes in language-specific regions of the dominant superior temporal lobe may be interposed between the word-percept and word-concept nodes. The proximity of these nodes in the fusiform gyrus suggests the intriguing possibility that semantic or conceptual representations of words may also be accessed directly within the ventral pathway. □

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A self-renewing multipotential stem cell in embryonic rat cerebral cortex

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NEUROECTODERM cells in the cortical ventricular zone generate many diverse cell types, maintain the ventricular zone during embryonic life and create another germinal layer, the subventricular zone, which persists into adulthood[1,2]. In other vertebrate tissues, including skin, intestine, blood and neural crest, stem cells are important in maintaining a germinal population and generating differentiated progeny[3–6]. By following the fates of single ventricular zone cells in culture, we show here that self-renewing, multipotential stem cells are present in the embryonic rat cerebral cortex. Forty per cent of these stem cells produced all three principal cell types of the central nervous system: neurons, astrocytes and oligodendrocytes. Stem cells constituted about 7% of cortical clones; in contrast, over 80% consisted of small numbers of neurons or glia. We suggest that multipotential stem cells may be the ancestors of other cortical progenitor cells that exhibit more limited proliferation and more restricted repertoires of progeny fates.

We analysed the development of 464 clones (Table 1) derived from embryonic day-12 (E12) and E14 cerebral cortices, which consist almost entirely of ventricular zone cells[1,2]. The majority (~80%) were small clones of neurons, defined by morphology and staining for neurofilament protein (Fig. 1a, b and Table 1). About 2% of single cells gave solely astrocytic or solely oligodendrocytic clones, judged by staining with antibodies against glial fibrillary acidic protein, which labels astrocytes (Fig. 1c and d) or with antibodies O4 and O1, which label oligodendrocytes[7] (Table 1). In contrast, about 7% of single cells continued to divide for weeks and produced hundreds of progeny. We examined the differentiated cell types present in these large clones and found that ~40% contained neurons, astrocytes and oligodendrocytes, revealing a common precursor for the three major classes of cells from the central nervous system (CNS) (Fig. 2). The remainder appeared to contain only neurons and astrocytes or only astrocytes and oligodendrocytes.

We investigated whether the cortical cells that generated large, multiple-type clones were stem cells. A defining characteristic of stem cells is their ability to renew themselves[3,4,8]. To test for self-renewal, we grew putative stem cell clones until they contained 20–30 cells (~4 days of culture), dissociated them and subcloned individual progeny into culture wells. If the initial cell had undergone self-renewal, some of the progeny would behave as stem cells, generating large, diverse clones. We found that this was the case in all eight clones that were subcloned. For example, for one 26-cell clone, ten subcloned progeny survived, seven generated clones of 1–5 cells, one gave 16 astrocytes, and two generated hundreds of progeny of multiple cell types (Fig. 3). This indicates that the initial cell was probably a stem cell capable of self-renewal.

The frequency of progenitor cell types was similar at E12 and E14; 76% of E12 and 81% of E14 cortical ventricular zone cells differentiated into one or two neurons. The frequency of stem cells at E12 and E14 was $7.4 \pm 7.5\%$ versus $7.3 \pm 1.5\%$ respec-

FIG. 1 Three of the clonal types. a, Phase micrograph of a single neuron, the most common product of the isolated ventricular zone cells. b, Larger neuron-only clone consisting of four progeny. c, Phase micrograph of a clone consisting solely of astrocytes. d, Astrocyte clone evident after staining with antiserum against GFAP, visualized with a fluorescein-labelled anti-rabbit antibody (Tago). Scale bar, 100 μm. Single E12–E14 cortical cells were mechanically dissociated in hibernation medium[27] and plated as one cell per Terasaki well by micromanipulation[19]. Culture medium was a serum-free medium (DMEM B27 plus N2 (Gibco)) which had been conditioned by cortical astrocytes and meningeal cells[28]. Culture wells contained sonicated membrane homogenates from C6 cells at 0.01 mg protein per ml. The C6 membrane supports division of isolated embryonic cortical ventricular zone cells[29]. Under these culture conditions about 85% of single E12–E14 cortical cells survived the first 24 h of culture, as judged by the presence of a live cell or its progeny in the culture wells.